

THE ESTIMATION OF THE ACTIVITY OF MICRO-ORGANISMS IN THE SOIL

BY

A. BURGES

Hartley Botanical Laboratories, University of Liverpool

Numerous qualitative and quantitative methods have been used to study the soil micro-organisms. Because of the difficulties involved, these methods are subject to substantial errors.

Two methods of estimating overall activity per unit volume of soil are discussed, (i) the measurement of oxygen uptake and carbon dioxide output and (ii) the measurement of enzyme activity in the soil.

Early studies in soil microbiology were concerned primarily with the isolation of the micro-organisms present, their identification and enumeration. Because of the difficulty of identifying large numbers of bacterial isolates, studies on this group were directed primarily towards counting the various populations either as a total bacterial count for any given soil, or at best, the counting of a relatively small number of easily recognized groups, such as sporeformers, cocci, rods, anaerobes, etc. On the other hand, studies of soil fungi were in the early days almost entirely taxonomic.

In more recent years, the mycologist has devoted more time to the estimation of fungal populations and the bacteriologist to the identification of isolates, and there has been a considerable move towards a synthetic approach to the study of soil micro-organisms. Insofar as fungi, actinomycetes and soil algae are concerned, there is now a very substantial volume of literature concerned with the species which are known to occur in soil (Burges, 1958) and many species lists for different soil types are available. There is still virtually no corresponding list for the bacterial populations, although a number of schools are making determined efforts to obtain such information.

As knowledge increased, it became clear that one wanted to know not only what species of organisms were present and how many of each

species, but also how active they were in controlling soil processes. This has led to a rapidly spreading interest in ways of estimating the activity of soil organisms. With bacteria, one can make an estimate of the probable number of bacterial cells present in a given volume of the soil being studied either by dilution plate techniques or by direct observation and one can use the total number of bacterial cells as a first approximation towards an estimate of the activity of this group. However, there is often a considerable discrepancy between counts obtained by dilution plate methods and by direct observation which strongly suggests that there are big differences between cells in their capacity to grow and correspondingly big differences in their physiological activity. Fungi present an even more difficult problem. There is now overwhelming evidence to indicate that the colonies of fungi which arise on a dilution plate are derived from spores (Warcup, 1955). Therefore, most estimates of the "number" of fungi in a given volume of soil represent little more than a count of the spores present. To some extent, this does give a measure of fungal activity, in that the number of spores present is a reflection of previous vegetative activity, but at this stage, we have no means of estimating the time between the counting of the spores and the vegetative activity which led to the accumulation of food reserves which made spore production possible. Many fungal spores are long-lived and the population of spores at any one time may well include long-lived, dormant spores which had been in the soil for months or more, as well as those recently formed. Again, however, one can use the estimate obtained from the spores as an approximate measure of fungal activity. Periodic counts show fluctuations in total spore numbers which can be associated either with changes which might be expected to influence fungal activity or with the addition of fresh supplies of food materials, the spore counts fluctuating with a lag of perhaps a few weeks on the factors which caused the change in fungal activity.

Within the last ten years there have been determined attempts to obtain other estimates of activity. Early work on measurements of oxygen uptake or carbon-dioxide output by the soil as a whole has been re-examined and a number of technical modifications developed. These are beginning to yield a great deal of information. The most widely-used technique is some form of Warburg apparatus in which a known quantity of soil is observed over a relatively short period and the oxygen uptake or carbon-dioxide output is measured. A number of special flasks have been designed (Parkinson & Coups, 1963) which allow the use of samples of soil, usually of about 5–10 g. and which allow the flask to be readily cleaned afterwards. Studies by Webley (1947), Rovira (1953) and others indicate that, in contrast with normal Warburg studies, there is little to be gained by shaking the flasks and most investigators have simply allowed the flasks to equilibrate in a constant temperature bath while stationary and to observe the gas exchange on the normal manometers. Some indications of the kind of results obtained are given in Table 1.

Detailed studies using this kind of technique have shown that it is a most valuable tool for rapid estimates of activity and for estimating the influence of certain specific factors, such as temperature, moisture

level and substrate concentration. Where more detailed studies have been undertaken, then more elaborate apparatus has been used, either because the investigator wished to use larger samples or because he wished to continue the investigation over a longer period. Examples of studies of this kind are those of Macura (1961) and Domsch (1963). As yet, there have

Table 1

Seasonal changes in oxygen uptake of the podzol horizons (Burgess, 1963)

| Soil horizon | Oxygen uptake ($\mu\text{l./g. dry wt. soil/5 hr.}$) | | | |
|-------------------------------|--|--------|--------|--------|
| | Spring | Summer | Autumn | Winter |
| A ₀ L | 391.7 | 342.8 | 2366.0 | 2206.0 |
| A ₀ F ₁ | 1277.0 | 255.4 | 1400.0 | 762.6 |
| A ₀ F ₂ | 319.7 | 157.6 | 245.2 | 234.9 |
| A ₀ H | 88.9 | 71.4 | 80.9 | 78.4 |
| A ₁ | 8.2 | 9.8 | 13.3 | 20.1 |
| A ₂ | 8.4 | 3.9 | 4.5 | 9.0 |
| B ₁ | 11.3 | 10.1 | 9.8 | 18.0 |
| B ₂ | 3.0 | 4.0 | 3.0 | 8.2 |
| C | 3.8 | 1.5 | 1.4 | 7.5 |

not been sufficiently extensive investigations of this kind to draw many conclusions. However, consideration of the information already available would suggest that moisture availability is a major factor in determining microbial activity and that, although extremes of temperature have a profound effect on soil microbiological activity, fluctuations between 2° and 30°C. lead to considerably smaller changes in overall activity than one would anticipate, in view of the known influence of temperature changes on enzymatic actions.

One of the most recent developments in the estimation of the activity of soil micro-organisms has involved the study of soil enzymes (Durand, 1965). Here the assumption has been made that if one could estimate total enzymatic activity of some key system, this would be the most satisfactory measurement of overall biological activity. This might be regarded as the biochemist's equivalent to the ecologist's concept of biomass.

The enzyme reaction which has been most studied is the formation of a coloured tetrazolium complex by dehydrogenase enzymes (Stevenson, 1959). There are two good reasons for the choice of this reaction. First is that the coloured complex formed can be extracted and its amount measured relatively rapidly photometrically. Second, dehydrogenase systems are fundamental in the normal metabolic processes of all organisms so far investigated and are therefore more likely to give a reliable estimate of activity than most other systems, which might be regarded as adaptive systems and therefore not a true measure of basic metabolic activity.

The use of soil enzymes as a measure of activity is still in its infancy. Preliminary studies suggest that the method works very well for bacterial populations in soils with relatively low clay content. Very considerable

difficulties attend the use of the method where the microbiological population is primarily fungal or where the soil has a very high clay content. Estimation of dehydrogenase activity in fungi is difficult, first because of the problems associated with the penetration of the cell wall by the tetrazolium dye and, second, because of the difficulty of extracting the coloured formazan complex from the undisturbed hyphae. Despite these difficulties, however, the method has very many potentialities and will repay further study.

In these days when there is very considerable interest in using the ecological concepts developed by higher plant and animal ecologists to soil microbiology, one could perhaps consider a count obtained either by the dilution plate method or by direct observation as being equivalent to the "standing crop" and the measurement of activity or turnover rate as equivalent to "productivity".

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